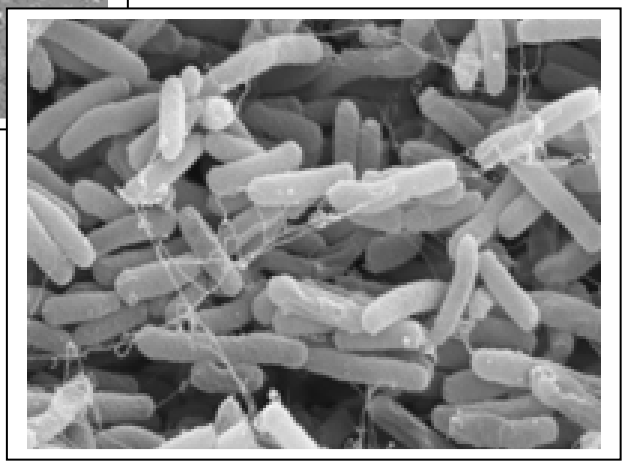
Granger Drain DNA Fecal Analysis Project

Sampling fresh bovine fecal material.



E. coli bacteria.

Funded through (1) a grant from the Environmental Protection Agency to the
South-Central Resource, Conservation and Development Council of the Natural Resources Conservation Service,
(2) the South Yakima Conservation District, and (3) the Washington State Department of Ecology.

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Executive Summary

In 2001, the Washington State Department of Ecology developed the *Granger Drain Bacteria Fecal Coliform Total Maximum Daily Load* (TMDL). To assist with initial implementation of the TMDL, the South Yakima Conservation District conducted a preliminary study of the sources of fecal coliforms in Granger Drain. Because of the extremely limited funding available to conduct this project it was intended to be an initial analysis not a definitive study.

The method used to identify sources was Microbial Source Tracking – analyzing the DNA of *Escherichia coli* from warm-blooded animals. Researchers have found that *E. coli* living within animals are generally species-specific. The DNA of *E. coli* identified in this project was compared against a library of approximately 65,000 isolates.

Fifty water samples were collected from May-September during the irrigation season of 2001 at one site near the base of the Granger Drain watershed. Of 146 DNA analyses conducted, the number of isolates identified were: 45 bovine, 21 avian, 16 human, 11 rodent, 11 deer/elk, 6 canine, 9 raccoon, 4 horse, 4 porcine, 2 sheep, 1 poultry, 1 feline, 1 muskrat, and 1 squirrel. Thirteen isolates were unidentified. Grouping the results into manageability, 49 percent of the isolates were from "manageable" sources such as livestock and failing septic systems and 42 percent were from "unmanageable" sources such as wildlife.

Despite the limitations due to the few number of samples analyzed, the following conclusions seem warranted from the data. (1) There are many sources of fecal coliforms to the Granger Drain, not just one or two. (2) The chances of finding bovine isolates in any given sample are higher than any other source. This suggests that, even with the significant past BMP implementation efforts and subsequent improvements in water quality, there is still a long ways to go with current efforts. (3) The roughly equal proportions between manageable and unmanageable sources suggests that determining what is "background" or uncontrollable is more important than initially considered in the TMDL.

Introduction

One of the challenges in implementing the *Granger Drain Fecal Coliform Bacteria Total Maximum Daily Load* (TMDL) is the uncertainty about the relative contributions of the various sources of fecal coliforms. One way to reduce this uncertainty is to identify sources of fecal coliforms through microbial source tracking.

Microbial source tracking compares the DNA of *Escherichia coli* from known types of sources against the DNA of *E. coli* from unknown sources in a surface water body. Since *E. coli* from warm-blooded animals have been found to be generally species-specific, when the DNA analyses match, the contributing species can be identified.

Setting and History. Granger Drain is an irrigation return drain that flows into the Yakima River. Documented problems have included elevated concentrations of suspended sediments, nutrients, bacteria, and DDT and its metabolites. The concentrations of these contaminants have decreased significantly over the past few years but are still at levels of concern.

The Granger Drain watershed includes approximately 18,000 acres of irrigated agricultural lands. Pastures, dairies, and irrigated crop production are major land uses. Crops grown in the watershed include corn, grapes, hops, alfalfa, apples, and asparagus. The predominant irrigation practice is rill (furrow). Slopes vary from 0 to over 30 percent. The steeper slopes tend to be on the sides of the valley above the Sunnyside canal where orchards, alfalfa, and corn are most common. Soils are most typically silt loam. The uppermost, non-irrigated part of the watershed is largely rangeland.

Past research has commonly attributed the high fecal coliform concentrations to the large numbers of cows within the watershed. There are approximately 43,000 dairy cows in Granger Drain watershed. The number of beef cows is unknown. There are estimated to be 12,000 people. The Washington State Department of Ecology states in the *Granger Drain Fecal Coliform Bacteria Total Maximum Daily Load Assessment and Evaluation: Final* (October 2001) that "the watershed's FC [fecal coliform] pollution is still assumed to be principally attributable to the numerous and concentrated livestock in the watershed, but indirectly instead of directly" (page 56). The TMDL identifies human waste and wildlife as "very minor" sources (page 59).

Project Objectives. The project objective was to obtain sufficient water and fecal source samples to estimate the proportions of sources of fecal contamination in Granger Drain during the irrigation season of 2001. Identification of fecal sources was to be based on major categories such as cows, humans, rodents, and waterfowl. Finer distinctions such as beef cows versus dairy cows were not attempted due to budget limitations.

Funding. In March 2001, the South Yakima Conservation District (SYCD) unexpectedly received a request to develop a project for potentially $5000 of funding from Natural Resources Conservation Service's South-Central Resource, Conservation and Development Council through an Environmental Protection Agency grant. This amount was insufficient to fund a fecal coliform DNA analysis project; however, there was great

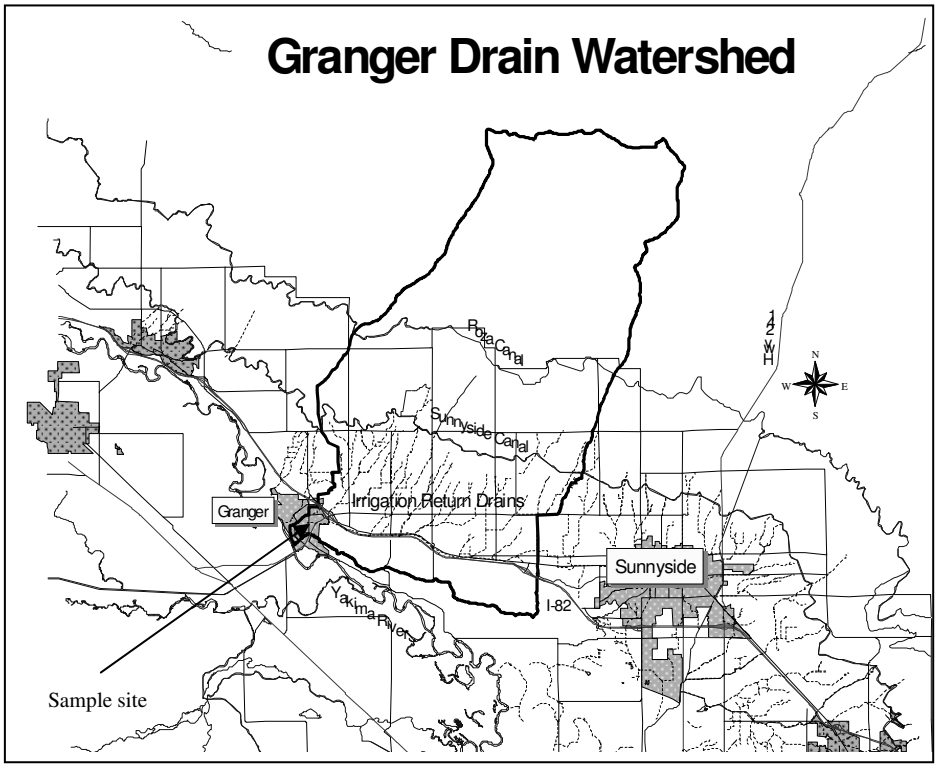
interest and support in the project from several agencies. To make the project happen, SYCD contributed $2500 and the Department of Ecology contributed $2000.

The Roza-Sunnyside Board of Joint Control (RSBOJC) agreed to conduct the water sampling with reimbursement for their laboratory analysis cost of $2000. Dr. Samadpour, with the Institute for Environmental Health, agreed to conduct 100 DNA analyses for $7500 despite the extremely fast project timeline. Total project expenditures were $9500, excluding SYCD staff time. Given this extremely limited budget, the intent of this project was to conduct only a preliminary analysis of the watershed, not a definitive study.

Sampling Design

Site Selection and Sampling Frequency. Only one site was sampled at approximately one-half mile upstream of the mouth of Granger Drain (see figure 1). This site, at the sheep barns, is used for water quality monitoring by the Roza-Sunnyside Board of Joint Control and the United States Geological Survey (USGS site 12505450 in STORET). The site represents the collection of all irrigation return drains in the watershed yet it is upstream from the town of Granger and possible "urban" influences. Although sampling several sub-drains was considered, Dr. Samadpour explained that since the budget severely restricted the number of samples to be taken, it would be best to take all the samples from one location. The one location should be most representative of the watershed or represent its most sensitive areas. The sheep barn site was the most representative of the entire watershed.

Figure 1. Map of the Granger Drain watershed



The site was sampled every two weeks from May – September 2001, for a total of 10 visits. Five replicate samples for DNA analysis were obtained concurrently with samples for RSBOJC's regular water quality monitoring. The non-irrigation season was not sampled for two reasons: (1) fecal coliform concentrations are significantly higher during the irrigation season; and (2) the Granger Drain TMDL focuses primarily on fecals transported by irrigation water. The original sampling design required at least two *E. coli* strains to be isolated from each of the 50 samples for a minimum of 100 *E. coli* strains. DNA analyses would be performed on each of these 100 *E. coli* strains.

Detailed sampling protocols are described in Appendix 1.

Variability. The typical temporal and spatial variability of fecal coliform concentrations in the Granger Drain system is high. For example, the geometric mean concentrations of fecals in different Granger Drain sub-basins during the irrigation season of 2000 ranged from 230-1,140 cfu/100 ml[1]. For this sampling effort, fecal coliform concentrations ranged from 330 to 1,300 cfu/100 ml. Variability of the types of sources is discussed below, in Results and Interpretation.

Source Sampling. To supplement the existing DNA isolate library of approximately 65,000 fingerprints, SYCD obtained 58 fecal samples from local sources. The number of each type of source sample was as follows: 18 dairy cows, 4 beef cows, 10 human, 8 horses, 9 dogs, and 9 cats. The human samples were taken from the treatment plant because, while it does not discharge to the Granger Drain, it does represent a well-mixed sample of many people. SYCD had intended to sample muskrats and a variety of birds but ran out of time. Dr. Samadpour estimated that the local source samples would increase the number of matches by perhaps only one percent.

Parameters. Samples for this project were analyzed using DNA ribotyping procedures. Because these samples were obtained at the same time as RSBOJC's regular monitoring, other data collected were: total suspended solids, turbidity, total Kjeldahl nitrogen, total phosphorus, nitrate/nitrite, fecal coliforms, temperature, dissolved oxygen, conductivity, pH, and flow.

Results and Interpretation

Out of the 50 water samples, two had no *E. coli* colonies. On the remaining 48 samples, DNA analyses were conducted on at least 3 of the *E. coli* colonies present in the sample, for a total of 146 DNA analyses.

Figures 2 and 3 show the distribution of the results.

[1] cfu/100 ml = colony forming unit per 100 milliliters. Sub-basin results from *Granger Drain Bacteria Fecal Coliform Total Maximum Daily Load Assessment and Evaluation: Final*, Washington State Department of Ecology, October 2000.

Figure 2. Distribution of fecal coliform sources in Granger Drain.

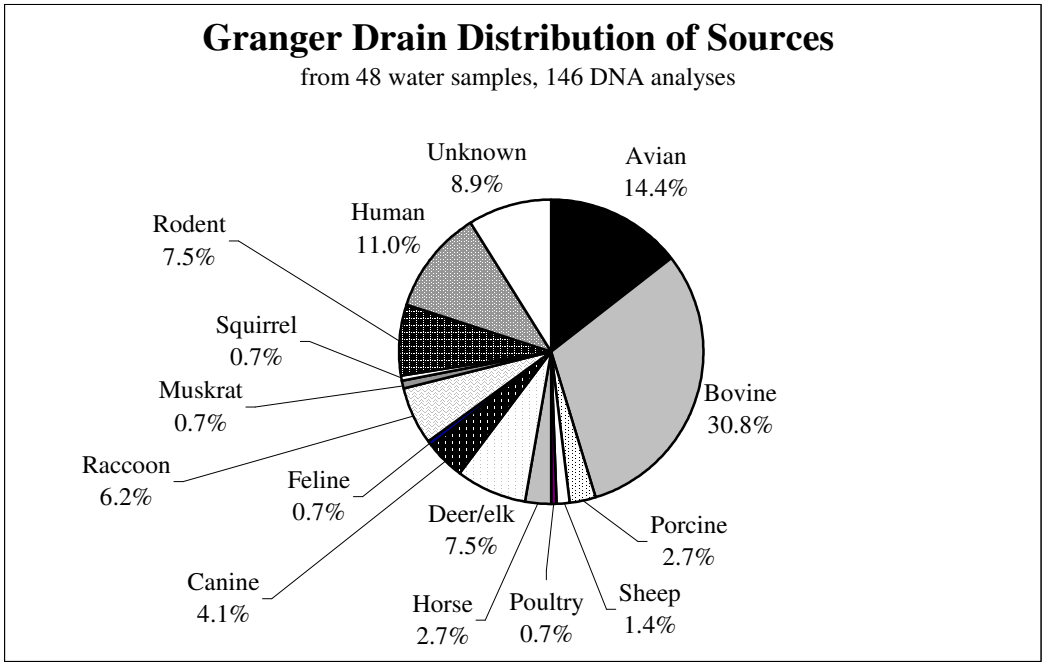
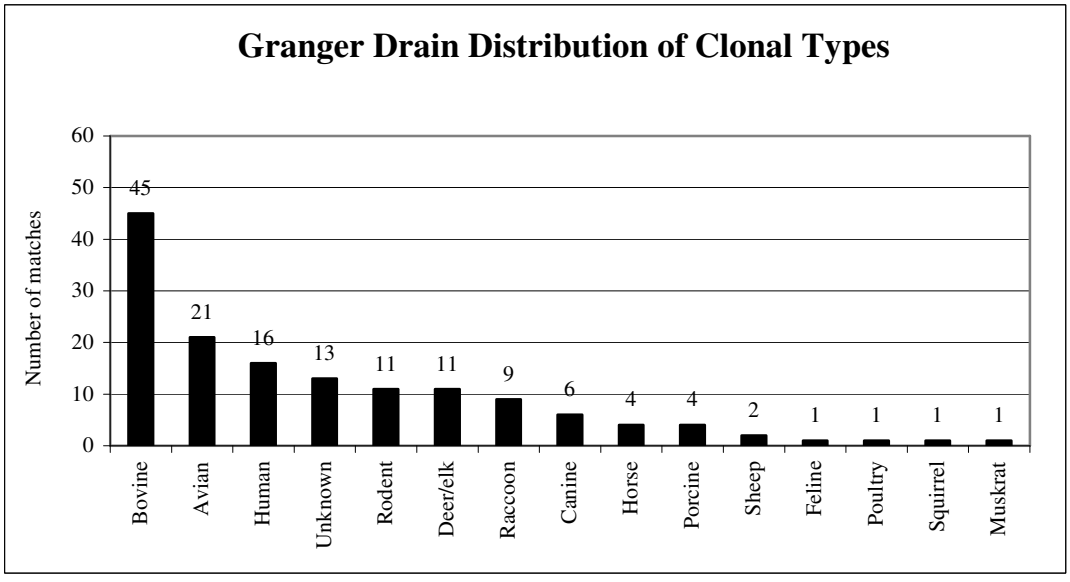
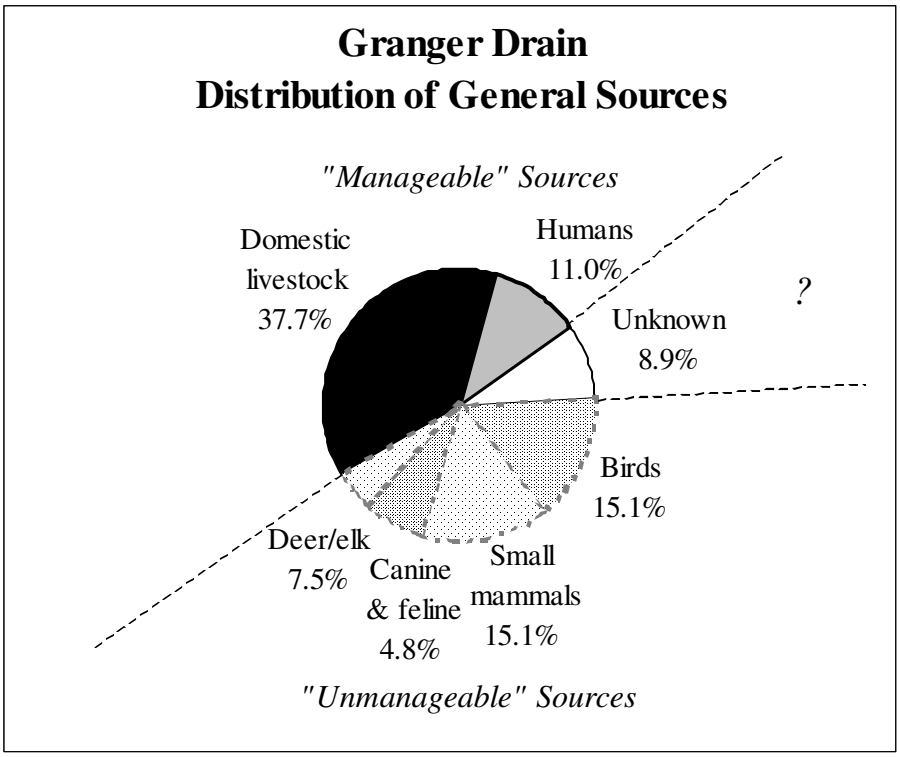


Figure 3. Distribution of clonal types.



One way to look at the types of sources in Granger Drain is whether or not the source is potentially "manageable" through best management practices or if it is "unmanageable," such as wildlife. In figure 4, domestic livestock and humans are considered "manageable" while the remaining sources are considered "unmanageable." Domestic livestock and humans account for 48.8% of the isolates while the remaining known sources account for 42.5% of the isolates.

Figure 4. Granger Drain distribution of general sources



The above figures look only at the total number of analyses. The totals do not reflect the differing fecal coliform concentrations found during sampling. As shown in figure 5, the types and proportions of isolates varied widely between sampling days.

Figure 5. Variability of fecal coliform concentrations and species identified in water samples.

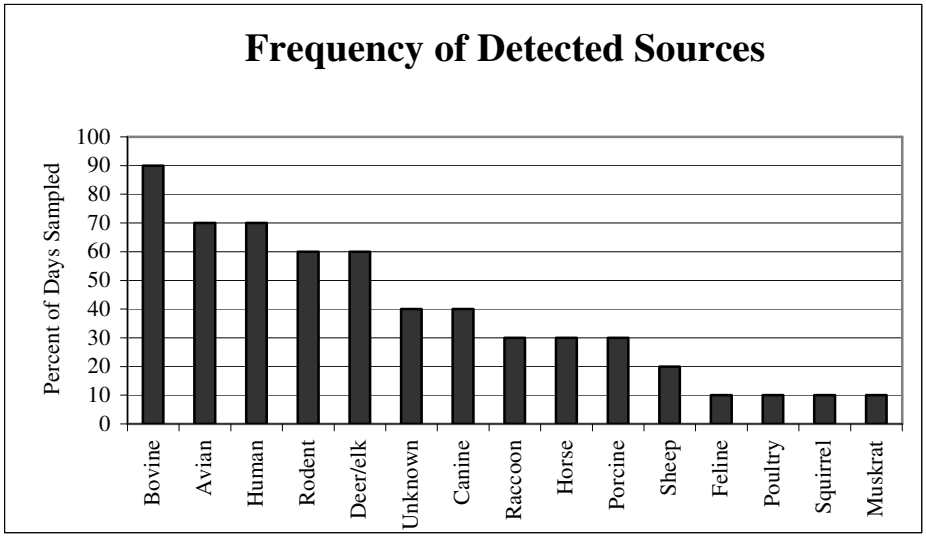
Date Sampled	Fecal coliform concentrations (colonies/100 mL)	Number and type of isolates found
5-16-01	1300	3 bovine, 5 avian, 2 rodent, 1 deer/elk, 1 horse
5-29-01	760	3 bovine, 1 avian, 5 human, 1 rodent, 3 deer/elk, 1 porcine, 1 unknown
6-12-01	1200	7 bovine, 1 human, 2 rodent, 2 raccoon, 1 poultry, 1 muskrat, 2 unknown
6-25-01	640	6 bovine, 1 avian, 2 human, 2 deer/elk, 1 raccoon, 1 canine, 1 porcine, 1 squirrel, 1 sheep
7-10-01	560	4 avian, 3 human, 3 rodent, 3 deer/elk, 1 canine, 1 raccoon
7-24-01	340	6 bovine, 4 avian, 1 human, 1 deer/elk, 2 canine, 1 raccoon
8-7-01	330	5 bovine, 4 unknown
8-21-01	750	3 bovine, 3 avian, 1 human, 1 rodent, 1 deer/elk, 2 canine, 1 raccoon, 1 horse, 2 porcine
9-5-01	330	7 bovine, 2 rodent, 6 unknown
9-17-01	350	5 bovine, 3 avian, 3 human, 2 horse, 1 sheep, 1 feline

There was no correlation between type of species and fecal coliform concentrations. Neither high nor low concentrations corresponded to any one species or "manageability" grouping.

Another way to look at the variability between samples was to determine how many samples had more than one of the same species found. Out of 48 samples, half had two isolates from the same species and half had all unique isolates. Appendix 2 lists the isolates found in each sample.

Species variability between days was very similar to the overall distribution of sources. Species found on more than one plate were found on two or more days, not disproportionately concentrated on certain days. The unknown isolates were the exception: unknown isolates were present on four days yet only two of these days accounted for 10 out of the 13 unknown isolates. Figure 6 shows the percentage of sampling days each species was identified.

Figure 6. Frequency of detected sources.



Quality Assurance. The Roza-Sunnyside Board of Joint Control's 90th percentile relative percent difference for fecal coliform measurements was 15.9% for the laboratory and 20.0% for the field from 1997-2001. These are low relative percent differences (high quality) for measuring biological parameters such as fecal coliforms.

The microbial source tracking method developed by Dr. Samadpour has been shown to determine the origin of *E. coli* strain with 96% specificity (Buck, F., 1997, Masters Thesis, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington). The accuracy of the method is further enhanced by eliminating clones that do not show host specificity from the MST database.

The reproducibility of the overall results, however, is unknown due to the difficulty in determining how well the analyzed colonies represent the diversity in the sampled surface water and thus the Granger Drain. For example, on a petri dish of up to 60 colonies, when only three are analyzed, how can one be assured that the colonies selected for DNA analysis actually represent the entire petri dish? The cost and complexity of attempting to answer this question was well beyond the scope of this project.

Conclusions

This project identified an unexpected diversity of sources of fecal coliforms in Granger Drain. Out of 146 DNA analyses conducted, the results were: 45 bovine, 21 avian, 16 human, 11 rodent, 11 deer/elk, 6 canine, 9 raccoon, 4 horse, 4 porcine, 2 sheep, 1 poultry, 1 feline, 1 muskrat, 1 squirrel, and 13 unknown.

Of the *E. coli* organisms identified, 49 percent were from "manageable" sources (cows, human, horse, sheep, porcine) and 42 percent were from "unmanageable" sources (wildlife and other animals that cannot be fenced in). This suggests that determining what is "background" or uncontrollable is more important than initially considered in the TMDL.

Bovine was the most frequent type of source identified. This suggests that, even with the significant past BMP implementation efforts (such as irrigation conversions in commercial crops, on-site manure management at dairies, irrigation water management, and sedimentation basins) and subsequent improvements in water quality, there is still a long ways to go with current efforts.

Appendix 1 Sampling Procedures

Water samples. Water samples were collected by Bill Rice, RSBOJC water quality specialist, and transported to their in-house, state-accredited laboratory for analysis within eight hours of collection. Sample collection, transport, and analysis were conducted in accordance with RSBOJC's existing, Ecology-approved QAPP.

Water samples were analyzed by the mFC method, SM 9222D (Standard Methods for Examination of Water and Wastewater, ASPH, 1997). All fecal coliform were boxed and shipped overnight to Dr. Samadpour's Laboratory, [(206) 543-5120] at 8279 Lake City Way NE, Seattle, WA 98115, for ribosomal RNA typing (referred to as DNA analysis throughout this document).

Source samples. Fresh animal fecal samples were collected aseptically into sterile containers provided by the Institute. The samples were shipped to Dr. Samadpour's laboratory by overnight mail on ice. Animal fecal samples were only collected when they were positively identified as belonging to a given animal species, and as soon as possible after deposition to prevent any contamination of the samples. No more than three samples were collected from the members of the same animal species from a single given location. Only a single sample was collected from an individual animal. All sample containers were labeled with the following information: sample type, host species, sample date and time, sample location, and sampler's initials. All the sample information was logged into a field log. Fecal samples were taken from throughout the watershed. At least 58 samples were taken. After collection, all samples were delivered to SYCD's office, where they were given a sample number and logged into the permanent sample logbook. The samples were kept refrigerated and were shipped to Dr. Samadpour's laboratory via overnight mail. Samples were stored for less than 4 days prior to shipping for analysis, except for one shipment exceeding the 4 day holding time.

<u>Environmental Health DNA analysis</u>

Two types of samples were sent to the Institute for Environmental Health: microbial plates from the RSBOJC laboratory and fecal source samples from SYCD. The Institute for Environmental Health's laboratory analysis includes:

a. Sample arrival and logging.
b. Isolation and purification of *E. coli* strains from water and fecal samples.
c. Growing pure cultures of *E. coli* strains for freezing (long-term storage), and isolation of DNA.
d. Restriction enzyme digestion and agarose gel electrophoresis of DNA samples.
e. Southern blot hybridization using radio labeled cDNA probe for rRNA genes.
f. Exposure of autoradiograms.
g. Analysis of the data.

a. Sample arrival and logging. All samples, upon arrival, were inspected for damage to sample containers or microbiological plates, and signs of contamination. Sample identifiers were also checked against the chain of custody papers. Samples were logged

into the log book noting the provider's sample identification number, provider ID, sample type, study ID, sample site, sample collection date, and sample arrival date.

b. Isolation and purification of E. coli strains from water and fecal samples. Water samples were received in the form of mFC plates, while fecal samples arrived in specimen containers. Fecal samples were plated on MacConkey agar and incubated at 35° C, overnight. The next day, 3-5 lactose fermenting, non-mucoied colonies were picked and replated on MacConkey agar for purification. Five non-mucoeid blue colonies were picked from mFC plates corresponding to each water sample and are plated on MacConkey agar for purification. At this stage, each of the colonies picked from a given sample had a provider Sample ID number and an accession letter. A single well-isolated non-mucoeid colony was picked from each MacConkey plate and was plated on Triptic Soy Agar, after overnight incubation at 35° C. Then each culture was tested by Spot indol test using appropriate positive and negative controls, and Indol positive cultures were further tested for the ability to utilize citrate using the Simon Citrate media. Indol positive, citrate negative colonies are identified as *E. coli* and are given isolate numbers.

c. Growing pure cultures of E. coli strains for freezing (long-term storage). A portion of each *E. coli* strain isolated from the samples was stored at -8°C, in a solution of nutrient broth plus 15% glycerol.

d. Isolation of DNA, restriction enzyme digestion and agarose gel electrophoresis of DNA samples. Genomic DNA was isolated from each *E. coli* strain using a standard protocol. All reagents and buffers were made according to formulas in the Institute's SOP. Reagents and buffers were tested for sterility. Every batch of restriction enzyme included two reactions with the positive control strain contained on two lanes on each gel. Agarose gel electrophoresis was conducted under standard conditions: agarose gel concentration and volume, buffer straight, pH, mA, V, and electrophoresis time were controlled for. Each agarose gel was assigned a number, and when more than one gel was run, the position of the first standard reference strain was changed in each gel (Lane 1 on the first gel was switched to the Nth lane on the Nth gel). After electrophoresis, gels were stained in ethidium bromide. Each set of two gels was stained in a single container, with one corner of the gel with the higher number being clipped. The label for each gel is also transferred to the staining container. After staining, each gel was then photographed and a hard copy of the print was labeled with the gel sheet parameters (containing the isolates numbers loaded on each lane, the enzyme used to cut the DNA, date, gel number, voltage, mA, gel strength, buffer strength, and electrophoresis time information) and kept in the gel book.

e. Southern blot hybridization using radio labeled cDNA probe for rRNA genes. Southern blotting was performed according to the protocol detailed in the Institute's SOP. After photography, each gel was returned to the same staining container. Gels were denatured for Southern blotting in the same container. Each blotting apparatus was performed in a separate container labeled with the gel number. Each membrane filter was labeled with the gel number, restriction enzyme designation, date, and technician's initials.

Data Reduction, Review, and Reporting. The only data received for this project were the final results from the Institute for Environmental Health. While we do not expect to ever need re-analysis of the samples, the *E. coli* plates are frozen and maintained in long-term storage at their laboratory.

3. Quality Control Procedures

Field QC Procedures. Bill Rice, Roza-Sunnyside Board of Joint Control, followed his existing QAPP procedures to collect the water samples. To collect the fecal source samples, the guidelines under part 5, Sampling Procedures, above, were followed.

Lab QC Procedures. To run the plates for mFC analysis, all relevant QC procedures in RSBOJC's current QAPP were followed. Dr. Samadpour has stated that QC procedures such as using a method blank, check standard, and duplicate analyses are not relevant to the DNA analyses. The Institute followed its internal SOP in processing and analyzing all samples received for this project.

Appendix 2: Sources Identified in Each Sample

Isolate #	Matched to	Provider Sample #	Sample Date
52549	avian	sycd1	5/16/2001
52550	avian	sycd1	5/16/2001
52551	raccoon	sycd1	5/16/2001
52552	avian	sycd2	5/16/2001
52553	bovine	sycd2	5/16/2001
52554	bovine	sycd2	5/16/2001
52555	bovine	sycd3	5/16/2001
52556	raccoon	sycd3	5/16/2001
52557	avian	sycd3	5/16/2001
52558	rodent	sycd4	5/16/2001
52559	raccoon	sycd4	5/16/2001
52560	deer/elk	sycd4	5/16/2001
52561	avian	sycd5	5/16/2001
52562	rodent	sycd5	5/16/2001
52563	horse	sycd5	5/16/2001
53247	rodent	sycd 1	5/29/2001
53248	human	sycd 1	5/29/2001
53249	human	sycd 1	5/29/2001
53250	human	sycd 2	5/29/2001
53251	human	sycd 2	5/29/2001
53252	human	sycd 2	5/29/2001
53253	deer	sycd 3	5/29/2001
53254	avian	sycd 3	5/29/2001
53255	bovine	sycd 3	5/29/2001
53256	deer/elk	sycd 4	5/29/2001
53257	porcine	sycd 4	5/29/2001
53258	deer	sycd 4	5/29/2001
53259	unknown	sycd 5	5/29/2001
53260	bovine	sycd 5	5/29/2001
53261	bovine	sycd 5	5/29/2001
53500	bovine	SYCD 1	6/12/2001
53501	bovine	SYCD 1	6/12/2001
53502	raccoon	SYCD 1	6/12/2001
53503	Bovine	SYCD 1	6/12/2001
53504	rodent	SYCD 2	6/12/2001
53505	muskrat	SYCD 2	6/12/2001
53506	unknown	SYCD 2	6/12/2001
53507	rodent	SYCD 3	6/12/2001
53508	bovine	SYCD 3	6/12/2001
53509	Bovine	SYCD 3	6/12/2001
53510	raccoon	SYCD 4	6/12/2001
53511	human	SYCD 4	6/12/2001
53512	poultry	SYCD 4	6/12/2001
53513	bovine	SYCD 5	6/12/2001
53514	unknown	SYCD 5	6/12/2001
53515	bovine	SYCD 5	6/12/2001
53889	human	SYCD #1	6/25/2001

53890	deer/elk	SYCD #1	6/25/2001
53891	sheep	SYCD #1	6/25/2001
53892	deer/elk	SYCD #2	6/25/2001
53893	avian	SYCD #2	6/25/2001
53894	raccoon	SYCD #2	6/25/2001
53895	bovine	SYCD #3	6/25/2001
53896	porcine	SYCD #3	6/25/2001
53897	human	SYCD #3	6/25/2001
53898	bovine	SYCD #4	6/25/2001
53899	coyote	SYCD #4	6/25/2001
53900	bovine	SYCD #4	6/25/2001
53901	Bovine	SYCD #4	6/25/2001
53902	bovine	SYCD #5	6/25/2001
53903	bovine	SYCD #5	6/25/2001
53904	squirrel	SYCD #5	6/25/2001
54091	raccoon	sycd 1	7/10/2001
54092	rodent	sycd 1	7/10/2001
54093	rodent	sycd 2	7/10/2001
54094	avian	sycd 2	7/10/2001
54095	rodent	sycd 2	7/10/2001
54096	avian	sycd 3	7/10/2001
54097	dog	sycd 3	7/10/2001
54098	human	sycd 3	7/10/2001
54099	human	sycd 4	7/10/2001
54100	deer/elk	sycd 4	7/10/2001
54101	deer/elk	sycd 4	7/10/2001
54102	human	sycd 4	7/10/2001
54103	deer/elk	sycd 5	7/10/2001
54104	avian	sycd 5	7/10/2001
54105	avian	sycd 5	7/10/2001
54958	canine	SYCD 1	7/24/2001
54959	avian	SYCD 1	7/24/2001
54960	bovine	SYCD 1	7/24/2001
54961	bovine	SYCD 1	7/24/2001
54962	avian	SYCD 2	7/24/2001
54963	bovine	SYCD 2	7/24/2001
54964	human	SYCD 2	7/24/2001
54965	fox	SYCD 3	7/24/2001
54966	avian	SYCD 3	7/24/2001
54967	deer	SYCD 3	7/24/2001
54968	raccoon	SYCD 4	7/24/2001
54969	avian	SYCD 4	7/24/2001
54970	bovine	SYCD 4	7/24/2001
54971	bovine	SYCD 5	7/24/2001
54972	bovine	SYCD 5	7/24/2001
55362	bovine	SYCD Granger Drain 3*	8/7/2001
55363	unknown	SYCD Granger Drain 3	8/7/2001
55364	unknown	SYCD Granger Drain 3	8/7/2001
55365	bovine	SYCD Granger Drain 3	8/7/2001
55366	unknown	SYCD Granger Drain 4	8/7/2001

* There were no *E. coli* colonies present on plates 1 or 2.

55367	bovine	SYCD Granger Drain 4	8/7/2001
55368	bovine	SYCD Granger Drain 5	8/7/2001
55369	bovine	SYCD Granger Drain 5	8/7/2001
55370	unknown	SYCD Granger Drain 5	8/7/2001
56742	deer	sycd 1	8/21/2001
56743	porcine	sycd 1	8/21/2001
56744	canine	sycd 1	8/21/2001
56745	porcine	sycd 2	8/21/2001
56746	raccoon	sycd 2	8/21/2001
56747	avian	sycd 2	8/21/2001
56748	bovine	sycd 3	8/21/2001
56749	horse	sycd 3	8/21/2001
56750	canine	sycd 3	8/21/2001
56751	avian	sycd 4	8/21/2001
56752	human	sycd 4	8/21/2001
56753	bovine	sycd 4	8/21/2001
56754	rodent	sycd 5	8/21/2001
56755	Bovine	sycd 5	8/21/2001
56756	avian	sycd 5	8/21/2001
56975	bovine	SYCD 1	9/5/2001
56976	unknown	SYCD 1	9/5/2001
56977	bovine	SYCD 1	9/5/2001
56978	rodent	SYCD 2	9/5/2001
56979	unknown	SYCD 2	9/5/2001
56980	bovine	SYCD 2	9/5/2001
56981	bovine	SYCD 3	9/5/2001
56982	unknown	SYCD 3	9/5/2001
56983	unknown	SYCD 3	9/5/2001
56984	bovine	SYCD 4	9/5/2001
56985	rodent	SYCD 4	9/5/2001
56986	unknown	SYCD 4	9/5/2001
56987	bovine	SYCD 5	9/5/2001
56988	bovine	SYCD 5	9/5/2001
56989	unknown	SYCD 5	9/5/2001
57273	avian	SYCD 1	9/17/2001
57274	avian	SYCD 1	9/17/2001
57275	sheep	SYCD 1	9/17/2001
57276	bovine	SYCD 2	9/17/2001
57277	horse	SYCD 2	9/17/2001
57278	bovine	SYCD 2	9/17/2001
57279	human	SYCD 3	9/17/2001
57280	avian	SYCD 3	9/17/2001
57281	horse	SYCD 3	9/17/2001
57282	human	SYCD 4	9/17/2001
57283	Bovine	SYCD 4	9/17/2001
57284	human	SYCD 4	9/17/2001
57285	bovine	SYCD 5	9/17/2001
57286	feline	SYCD 5	9/17/2001
57287	bovine	SYCD 5	9/17/2001